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Cell Signalling: IP_3 Receptors Channel Calcium into Cell Death

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There is substantial evidence that Ca^{2+} fluxes occur during most forms of apoptosis, and that inhibiting such fluxes protects cells from death. IP_3 receptors — ligand-gated channels that release Ca^{2+} from intracellular stores — are emerging as key sites for regulation by pro- and anti-apoptotic factors.

The cytosolic Ca^{2+} concentration in resting cells is maintained at low levels (~100 nM) by enzymes that translocate Ca^{2+} ions across the plasma membrane or into intracellular stores. Ca^{2+} uptake into mitochondria is another important way of removing cytosolic Ca^{2+} ions: by sequestering Ca^{2+} ions, mitochondria can modulate the kinetics and spatial dimensions of cellular Ca^{2+} signals [1]. Elevation of Ca^{2+} within the mitochondrial matrix activates citric acid cycle enzymes and thereby stimulates ATP production [2]. The cytosolic Ca^{2+} level is increased physiologically when cells are challenged with stimuli such as hormones, growth factors, depolarisation or mechanical deformation. The Ca^{2+} ions can be released from intracellular stores, enter the cell across the plasma membrane, or a combination of the two. Cells employ a diverse range of messengers and channels to access these sources [3]. For mobilising Ca^{2+} stores, inositol 1,4,5-trisphosphate (IP_3) receptors are a principal route in almost all cell types. The release of Ca^{2+} via IP_3 receptors stimulates activities critical for life, such as post-fertilization Ca^{2+} oscillations [4], but under some conditions IP_3 receptor-mediated Ca^{2+} signals are subverted to cause cell death.

IP_3 receptors are large (~1200 kDa) tetrameric proteins, each subunit of which projects an amino-terminal domain into the cytoplasm, their membrane-spanning carboxy-terminal regions forming an integral Ca^{2+} channel. IP_3 binding by the amino-terminal domains causes a conformational change that promotes channel opening. Between the IP_3 binding site and the transmembrane regions is a large stretch of amino acids where a significant proportion of regulatory interactions occur. Although IP_3 is necessary to open native IP_3 receptors, activation of these channels is complex and their open probability actually depends on the ambient Ca^{2+} concentration. Up to ~500 nM, Ca^{2+} works synergistically with IP_3 to activate IP_3 receptors. At higher concentrations, cytosolic Ca^{2+} inhibits IP_3 receptor opening. The inhibition of IP_3 receptors by Ca^{2+} is thought to be a crucial mechanism for terminating channel activity and thus preventing pathological Ca^{2+} rises.

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IP_3 Receptors in Apoptosis

A number of studies have shown that reducing IP_3 receptor expression inhibits apoptosis. For example, DT40 cells (a chicken B lymphoma cell line) in which IP_3 receptor expression was prevented were not only deficient in IP_3-mediated Ca^{2+} signalling, but also substantially resistant to the apoptosis normally induced in response to B-cell receptor activation [5]. Similarly, Jurkat cells with reduced expression of IP_3 receptors showed only a modest activation of caspases 3 and 9 following engagement of the CD3 component of the T-cell receptor [6]. Caspases are aspartate-directed cysteine proteases responsible for a cascade of events that culminate in cell death.

Three IP_3 receptor isoforms have been cloned and splice variants have been described, leading to the possibility that heteromultimeric channels are assembled with distinctive properties relating to their subunit content. At present, it is not clear whether different IP_3 receptor isoforms have an equivalent role in apoptosis. In the studies using DT40 cells, there appeared to be redundancy between different IP_3 receptor isoforms. Cells where all three isoforms were knocked out showed the least death compared to cells missing either a single isoform or pairs of isoforms [5]. In contrast to this, some studies have shown that reduction of either type 1 or type 3 IP_3 receptor abrogates apoptosis.

How Does Ca^{2+} Cause Apoptosis?

Transfer of Ca^{2+} between intracellular stores and mitochondria provides physiological control of respiration. But this Ca^{2+} cycle can also lead to cell death. If the matrix Ca^{2+} level rises too high, or if normal Ca^{2+} signals occur concurrently with production of molecules such as arachidonate or ceramide, then deleterious changes in mitochondrial structure may occur. In particular, mitochondria can swell and rupture or undergo permeability transition, thereby releasing several pro-apoptotic factors into the cytoplasm, such as cytochrome C, second mitochondrial activator of caspases (SMAC/Diablo) or apoptosis-inducing factor (AIF) [7]. This leads to the generation of the 'apoptosome' and activation of caspases from inactive zymogens. It is well established that Ca^{2+} released through IP_3 receptors is sequestered by mitochondria [2]. Furthermore, it has been demonstrated that the flow of Ca^{2+} specifically from IP_3 receptors can cause mitochondrial permeability transition and activate the apoptotic cascade [8].

In addition to directly feeding mitochondria with Ca^{2+}, activation of IP_3 receptors can stimulate a variety of Ca^{2+}-sensitive enzymes that engage other apoptotic mechanisms. One clear target of IP_3-mediated Ca^{2+} signals is the phosphatase calcineurin. This enzyme has many substrates, but in particular it can dephosphorylate the pro-apoptotic protein Bad causing it to translocate from the cytosol to mitochondria and thereby stimulate cytochrome C release. T cells

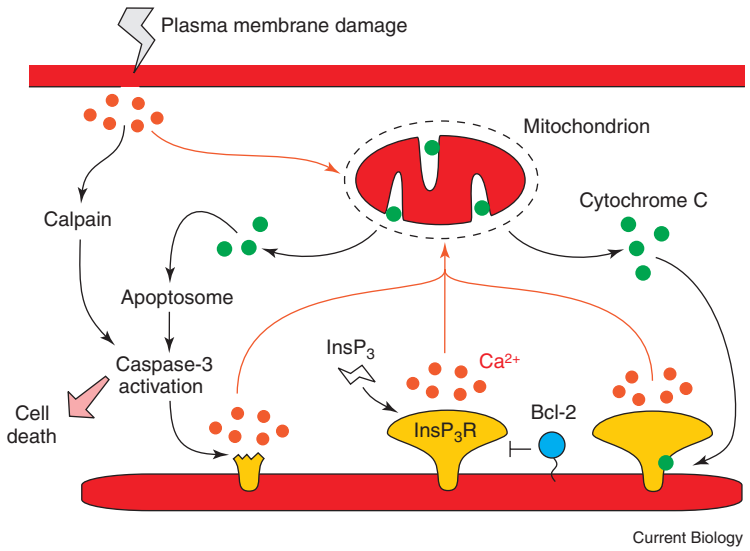

Figure 1. Some of the interactions that lead to recruitment of IP_3 receptors during apoptosis.

The positive feedback between IP_3 receptor-mediated Ca^{2+} release and mitochondria underlies the generation of Ca^{2+} signals that accelerate the rate of cell death. The apoptosis-inducing cycle of Ca^{2+} between IP_3 receptors and mitochondria can be initiated by a variety of mechanisms, including non-specific entry of Ca^{2+} following membrane damage.

deficient in IP_3 receptors were found to be resistant to a variety of apoptotic stimuli; however, expression of a constitutively active form of calcineurin or pharmacological elevation of Ca^{2+} could by-pass the need for IP_3-induced Ca^{2+} release and restore cell death [9].

How Are IP_3 Receptors Activated during Apoptosis?

The physiological way of activating IP_3 receptors, of course, is to provoke intracellular production of IP_3. Natural IP_3-generating agonists can induce apoptosis under some conditions. In the case of immature B cells, for example, apoptosis can be induced by IP_3-mediated Ca^{2+} signals arising from activation of the B-cell receptor. This is essential to establish immunological self-tolerance. In contrast, with mature B cells, B-cell receptor engagement provokes proliferation and antibody production. In a similar vein, application of an IP_3-generating agonist to mouse embryonic fibroblasts enhanced apoptosis when it was applied concurrently with a pro-apoptotic stimulus such as arachidonate [10].

Recent studies have demonstrated that IP_3 receptors can also be activated during apoptosis independently of the production of IP_3. In the case of type 1 IP_3 receptors, this may occur following proteolysis by activated caspase 3. The type 1 IP_3 receptor isoform has a single highly conserved DEVD cleavage site, which is caspase 3 specific. Truncation of IP_3 receptors by caspase 3 at this position removes a large portion of the protein, including the amino-terminal IP_3-binding region and other regulatory domains. The part that remains in the endoplasmic reticulum (ER) membrane is a constitutively active channel that continuously leaks Ca^{2+} [11].

The critical role of IP_3 receptor cleavage by caspase 3 in apoptosis was recently demonstrated by Assefa *et al.* [12]. They used DT40 cells in which all three IP_3 receptor isoforms had been knocked out so that the cells are insensitive to staurosporine: exogenous expression of normal type 1 IP_3 receptors — but not mutant receptors that cannot be cut by caspase-3 — restored sensitivity to staurosporine-induced apoptosis [12]. Furthermore, expression of the 'channel only' domain produced following proteolysis of IP_3 receptors in the triple knock-out DT40 cells also sensitised them to apoptosis.

Many pro-apoptotic stimuli, such as staurosporine, cause Ca^{2+} signals as part of their mechanism of killing. Buffering the Ca^{2+} rise can prevent, or at least delay, the onset of apoptosis [13]. The study by Assefa *et al.* [12] suggests that the Ca^{2+} elevation occurs because of IP_3 receptor cleavage. Consistent with this, the DT40 cells expressing normal type 1 IP_3 receptors, but not the caspase-resistant mutant, displayed increased cytosolic Ca^{2+} following staurosporine addition. The Ca^{2+} rise was blocked by a specific caspase 3 inhibitor [12]. These data reveal that type 1 IP_3 receptors are critical targets for caspase cleavage, and the subsequent unregulated release of Ca^{2+} accelerates cell death.

In an alternative model for IP_3 receptor activation during apoptosis that is not reliant on caspase cleavage, cytochrome C released from mitochondria is suggested to control Ca^{2+} movement through IP_3 receptors. Cytochrome C was shown to bind IP_3 receptors and reduce the Ca^{2+}-dependent inhibition of channel opening [14]. Within a cell undergoing apoptosis, this generates a positive feedback loop, whereby Ca^{2+} stimulates cytochrome C release and vice versa. The significance of this amplification mechanism was demonstrated by expressing a peptide corresponding to the carboxy-terminal portion of IP_3 receptors, where cytochrome C binds: this peptide buffers cytochrome C as it emerges from mitochondria and prevents its interaction with functional IP_3 receptors. As predicted, the carboxy-terminal IP_3 receptor fragment acted in a dominant-negative fashion and substantially reduced both the amplitude of Ca^{2+} signals and release of cytochrome C following addition of staurosporine [14].

These studies all point to a crucial role of IP_3 receptors in the generation of Ca^{2+} signals that drive cells into apoptosis. But it is unclear which model accounts for the activation of Ca^{2+} release. The schemes involving caspase 3 cleavage and cytochrome C binding appear to be mutually exclusive, in that IP_3 receptors cleaved by caspase 3 are constitutively open and so presumably do not require cytochrome C

binding. On the other hand, cytochrome C only regulates channels that can still bind IP_3, but caspase 3 cleavage removes the IP_3 binding site. It is possible that caspase 3 and cytochrome C have a synergystic action, work sequentially or target different IP_3 populations.

IP_3 receptors are by no means the only mechanism by which cellular Ca^{2+} can be elevated during cell death. Several other types of channel are known to transport Ca^{2+} and promote apoptosis. In particular, members of the TRPM family — melastatin-related transient receptor potential cation channels — underlie Ca^{2+} fluxes and cell death in neurons. And IP_3 receptors are not the only targets for caspases. It has been demonstrated that the Ca^{2+}ATPases on the plasma membrane are cleaved by caspases, thus preventing normal homeostasis and consequently causing Ca^{2+} elevation [15]. Damage to cellular membranes can cause a leak of Ca^{2+} into the cytoplasm, which often leads to the activation of Ca^{2+}-dependent cysteine endopeptidases known as calpains. These proteases have been shown to activate caspases, cause cytochrome C release and promote cell death.

Interaction with the Anti-Apoptotic Protein Bcl-2

As Ca^{2+} signals emanating from the ER via IP_3 receptors are critical for induction of cell death, regulating Ca^{2+} release is an obvious way for cells to control apoptotic signalling. Consistent with this, the anti-apoptotic protein Bcl-2 has been shown to reduce ER Ca^{2+} release, decrease mitochondrial Ca^{2+} uptake and abrogate cell death. Bcl-2 is a small integral membrane protein that localizes to both mitochondria and the ER [16] and so is present at the key locations where Ca^{2+} transport occurs. It has been proposed that Bcl-2 decreases the Ca^{2+} content of the ER and thereby reduces the flux of Ca^{2+} from IP_3 receptors to mitochondria. But whilst it is clear that manipulating ER luminal Ca^{2+} concentration can alter the sensitivity of cells to apoptosis [17], whether Bcl-2 works via this mechanism is controversial [18].

An alternative scheme by which Bcl-2 might regulate IP_3 receptor activity was recently highlighted by the observation of a direct interaction between these proteins [19]. Expression of Bcl-2 in a T-cell line was found to inhibit Ca^{2+} signals in response to a membrane-permeant IP_3 analogue, without altering the ER Ca^{2+} content. Furthermore, the open probability of purified IP_3 receptors incorporated into planar lipid bilayers was reduced by addition of recombinant Bcl-2. The direct coupling of these proteins within cells was substantiated using bi-directional co-immunoprecipitation of IP_3 receptors (isoforms 1 and 3) and Bcl-2. Where Bcl-2 binds to IP_3 receptors, and how it alters channel activity, are not known. Although the experiments in planar lipid bilayers suggest that the interaction is direct, it is plausible that within cells Bcl-2 also acts to scaffold other proteins to IP_3 receptors. For example, it has been proposed that Bcl-2 docks calcineurin with IP_3 receptors [20]. By regulating the phosphorylation status of IP_3 receptors, calcineurin could modulate Ca^{2+} release.

Without the participation of IP_3 receptors, cells are either resistant to pro-apoptotic stimulation or cell death is dramatically slowed. The precise mechanism by which IP_3 receptors are recruited during apoptosis is uncertain. It is clear, however, that Ca^{2+} accelerates the rate of apoptosis, and that IP_3 receptors provide an amplification mechanism by which sustained Ca^{2+} signals can arise (Figure 1).

References

1. Tinel, H., Cancela, J.M., Mogami, H., Gerasimenko, J.V., Gerasimenko, O.V., Tepikin, A.V., and Petersen, O.H. (1999). Active mitochondria surrounding the pancreatic acinar granule region prevent spreading of inositol trisphosphate-evoked local cytosolic Ca^{2+} signals. EMBO J. *18*, 4999-5008.
2. Rizzuto, R., Duchen, M.R., and Pozzan, T. (2004). Flirting in little space: the ER/mitochondria Ca^{2+} liaison. Sci STKE 2004, re1.
3. Bootman, M.D., Berridge, M.J., and Roderick, H.L. (2002). Calcium signalling: more messengers, more channels, more complexity. Curr. Biol. *12*, R563-565.
4. Halet, G., Marangos, P., Fitzharris, G., and Carroll, J. (2003). Ca^{2+} oscillations at fertilization in mammals. Biochem. Soc. Trans. *31*, 907-911.
5. Sugawara, H., Kurosaki, M., Takata, M., and Kurosaki, T. (1997). Genetic evidence for involvement of type 1, type 2 and type 3 inositol 1,4,5-trisphosphate receptors in signal transduction through the B-cell antigen receptor. EMBO J. *16*, 3078-3088.
6. Tantral, L., Malathi, K., Kohyama, S., Silane, M., Berenstein, A., and Jayaraman, T. (2004). Intracellular calcium release is required for caspase-3 and -9 activation. Cell Biochem. Funct. *22*, 35-40.
7. Orrenius, S., Zhivotovsky, B., and Nicotera, P. (2003). Regulation of cell death: the calcium-apoptosis link. Nat. Rev. Mol. Cell Biol. *4*, 552-565.
8. Szalai, G., Krishnamurthy, R., and Hajnoczky, G. (1999). Apoptosis driven by IP3-linked mitochondrial calcium signals. EMBO J. *18*, 6349-6361.
9. Jayaraman, T., and Marks, A.R. (1997). T cells deficient in inositol 1,4,5-trisphosphate receptor are resistant to apoptosis. Mol. Cell Biol. *17*, 3005-3012.
10. Scorrano, L., Oakes, S.A., Opferman, J.T., Cheng, E.H., Sorcinelli, M.D., Pozzan, T., and Korsmeyer, S.J. (2003). BAX and BAK regulation of endoplasmic reticulum Ca^{2+}: a control point for apoptosis. Science *300*, 135-139.
11. Nakayama, T., Hattori, M., Uchida, K., Nakamura, T., Tateishi, Y., Bannai, H., Iwai, M., Michikawa, T., Inoue, T., and Mikoshiba, K. (2004). The regulatory domain of the inositol 1,4,5-trisphosphate receptor is necessary to keep the channel domain closed: possible physiological significance of specific cleavage by caspase 3. Biochem. J. *377*, 299-307.
12. Assefa, Z., Bultynck, G., Szlufcik, K., Nadif Kasri, N., Vermassen, E., Goris, J., Missiaen, L., Callewaert, G., Parys, J.B., and De Smedt, H. (2004). Caspase-3-induced truncation of type 1 inositol trisphosphate receptor accelerates apoptotic cell death and induces inositol trisphosphate-independent calcium release during apoptosis. J. Biol. Chem. in press.
13. Kruman, I., Guo, Q., and Mattson, M.P. (1998). Calcium and reactive oxygen species mediate staurosporine-induced mitochondrial dysfunction and apoptosis in PC12 cells. J. Neurosci. Res. *51*, 293-308.
14. Boehning, D., Patterson, R.L., Sedaghat, L., Glebova, N.O., Kurosaki, T., and Snyder, S.H. (2003). Cytochrome c binds to inositol (1,4,5) trisphosphate receptors, amplifying calcium-dependent apoptosis. Nat. Cell Biol. *5*, 1051-1061.
15. Schwab, B.L., Guerini, D., Didszun, C., Bano, D., Ferrando-May, E., Fava, E., Tam, J., Xu, D., Xanthoudakis, S., Nicholson, D.W., Carafoli, E., and Nicotera, P. (2002). Cleavage of plasma membrane calcium pumps by caspases: a link between apoptosis and necrosis. Cell Death Differ. *9*, 818-831.
16. Cory, S., Huang, D.C., and Adams, J.M. (2003). The Bcl-2 family: roles in cell survival and oncogenesis. Oncogene *22*, 8590-8607.
17. Pinton, P., Ferrari, D., Rapizzi, E., Di Virgilio, F., Pozzan, T., and Rizzuto, R. (2001). The Ca^{2+} concentration of the endoplasmic reticulum is a key determinant of ceramide-induced apoptosis: significance for the molecular mechanism of Bcl-2 action. EMBO J. *20*, 2690-2701.
18. Distelhorst, C.W., and Shore, G.C. (2004). Bcl-2 and calcium: controversy beneath the surface. Oncogene *23*, 2875-2880.
19. Chen, R., Valencia, I., Zhong, F., McColl, K.S., Roderick, H.L., Bootman, M.D., Berridge, M.J., Conway, S.J., Holmes, A.B., Mignery, G.A., Velez, P., and Distelhorst, C.W. (2004). Bcl-2 functionally interacts with inositol 1,4,5-trisphosphate receptors to regulate calcium release from the ER in response to inositol 1,4,5-trisphosphate. J. Cell Biol. *166*, 193-203.
20. Erin, N., Lehman, R.A., Boyer, P.J., and Billingsley, M.L. (2003). *In vitro* hypoxia and excitotoxicity in human brain induce calcineurin-Bcl-2 interactions. Neuroscience *117*, 557-565.